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Secondary outputs of alpha$_1$-antitrypsin deficiency targeted detection programme

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Summary
Targeted detection programmes are recommended to identify subjects affected by severe alpha$_1$-antitrypsin deficiency (AATD). Guidelines are available to address physicians towards subjects at high risk for AATD. We wanted to investigate the clinical characteristics of subjects enrolled in the programme, who result as not being affected by severe AATD; this information is not available in the present literature. We elaborated data contained in the questionnaires accompanying the samples of 2127 Italian subjects submitted for AATD detection in a period spanning 11 years (1996–2006).
A total of 588 subjects were eligible to enter this study: PI*MM subjects and subjects with intermediate AATD, referred for lung disease, were characterised by a relatively young mean age, and a high proportion (31.2% and 28.6%, respectively) were never smokers. Fifty percent or more had symptoms of chronic bronchitis, but without obstruction. Only a minority belonged to most severe GOLD stages. The mean levels of AAT varied as a function of the presence or absence of airflow obstruction in intermediate AATD subjects, but not in PI*MM. Individuals enrolled in AATD detection programmes represent an interesting cohort both for public health and research purposes.

Introduction

Alpha$_1$-antitrypsin deficiency (AATD) is an inherited condition characterised by reduced plasma levels of alpha$_1$-antitrypsin (AAT), and increased risk of developing chronic, disabling pulmonary and liver disease.[1] The highest prevalence of AATD is recorded in northwestern European countries and in North America, and is linked to the commonest deficiency variant, referred to as PI*Z (14q32.1; SERPINA1 gene; ^{342}Glu→Lys).[2]

Development of national registries for AATD, based on detection programmes, is considered a critical issue to make advances in the understanding of the epidemiology and natural course of the disorder.[3] Strategies to detect

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individuals affected by AATD vary; the most common approach used by the majority of European national detection programmes is referred to as ''targeted detection'', and submits individuals at high risk for AATD to laboratory diagnosis. The ATS/ERS recently published a joint statement on AATD, indicating that individuals at high risk fall into the following categories[4]:

- individuals with pulmonary emphysema/COPD characterised by early onset, familial clustering, absence of detectable risk factors, pulmonary bibasilar hyperlucency;
- individuals with liver disease characterised by the absence of detectable risk factors and familial clustering;
- individuals with family members diagnosed as affected by AATD (family screening);
- *miscellanea* (ANCA-associated vasculitis, necrotizing panniculitis, casual detection of reduced plasma levels of AAT).

As a result of disseminated screening programmes, large cohorts of AATD individuals have been identified during the last decade. As an example, the Alpha One International Registry recently reported the largest series of AATD individuals, detected and enrolled according to a perspective model since 1999.[5] Besides the identification of subjects presenting with severe AATD, which is understandably the major output of the detection programmes, there are even larger cohorts of individuals theoretically at risk investigated and resulting as carriers of intermediate AATD deficiency (PI*MZ or PI*MS) or without deficiency variants. With the exception of subjects with intermediate AATD detected during a family screening, only little attention, if any, has been paid so far to the clinical characteristics of these subjects. In the present paper we describe the secondary outcomes of a programme aimed at detecting individuals affected by severe AATD. Although the programme was successfully designed for this aim, as elsewhere reported,[6,7] we nevertheless feel that it reveals other relevant issues worth reporting and discussing.

Methods

The organisation, structure, and flow of samples and questionnaires for diagnosis of AAT status have been already described in detail.[6] Briefly, paper filters and questionnaires were distributed mostly among general practitioners, internal medicine physicians, and pulmonologists nationwide since 1996. Until 2002, laboratory diagnosis for AAT status was performed on dried blood spots (DBS) from filters by means of immunodiffusion, to determine the AAT level, and isoelectric focusing (IEF).[6] Subsequently, genotyping and nephelometric determination of AAT levels were introduced.[8,9]

Based on data retrieved from the questionnaires, diagnosed subjects were assigned to one of the following ''reason for diagnosis'' categories (see the Introduction for details):

- lung disease,
- liver disease,
- family screening,
- miscellanea.

Variables (age, gender, smoking habit, pre-bronchodilator FEV_1 and FVC) were retrieved from the questionnaires, and COPD patients were classified according to revised GOLD parameters.[10] Statistical analysis was performed with STATISTICA for Windows (StatSoft, Bedford, UK, 2002). Comparison between means were performed with analysis of variance, using the Scheffè test for post hoc comparison. Association between category variables were compared with Fisher's exact test.

Results

In a period spanning 11 years (1996–2006), 534 Italian physicians contributed to our AATD detection programme, by shipping at least one sample. A total of 2127 samples (paper filter and questionnaire) were received and processed. The initial stratification, reported in Table 1, was performed according to the resulting genotype. Clinical characteristics of individuals with the severe AATD PI*ZZ and PI*SZ are described elsewhere[6] as well as those carrying the severe AATD PI*RR and the intermediate AATD PI*MR[7]; thus, these subjects ($N = 279$) are not included in this paper. We did not includ also the unique PI*SS patient.

Next, remaining individuals were stratified according to the reason for screening as detailed by the physicians providing the sample. From this analysis, we excluded 336 subjects because of missing information regarding the reason for screening. The large majority of samples belonged to individuals diagnosed with pulmonary disease (62%) followed by samples collected for family screening (29%). In 9% of samples (145 out of 1511), patients had been diagnosed with liver disease or other pathologies. Nevertheless, for the purposes of this paper, we further evaluated only subjects with complete questionnaires: 508 out of 933 samples submitted for pulmonary disease and 141 out of 433 samples submitted for family screening. Moreover, among patients diagnosed with pulmonary disease, a further 61 subjects, after careful review of questionnaires, were excluded, since the proposed diagnosed disagreed with normal lung function and absence of symptoms (cough and phlegm), as reported by physicians themselves (Table 2).

Table 1 Stratification of subjects investigated as a function of genotypic class.

Genotype	*N*	%
PI*MM	1326	62.4
PI*MS	140	6.6
PI*MZ	381	17.9
PI*MR	53	2.5
PI*SS	1	0.05
PI*SZ	39	1.8
PI*ZZ	155	7.3
PI*RR	32	1.5
Total	2127	

Table 2 Subjects stratified as a function of ''reason for screening'' for each genotypic class.

Reasons for screening	Total samples		PI*MM		PI*MS		PI*MZ	
	Submitted *N* (%)	Evaluated	Submitted *N* (%)	Evaluated	Submitted *N* (%)	Evaluated	Submitted *N* (%)	Evaluated
Lung disease	933 (62)	447	586 (73)	349	66 (68)	36	98 (29)	62
Family screening	433 (29)	141	142 (18)	50	22 (23)	5	206 (61)	86
Liver disease	67 (4)		25 (3)		4 (4)		22 (6)	
Miscellanea	78 (5)		45 (6)		5 (5)		14 (4)	

Table 3 Clinical characteristics of subjects diagnosed with lung disease.

	PI*MM ($N = 349$)	Intermediate (PI*MS+PI*MZ) AATD ($N = 98$)
Male (%)	274 (78.5)	67 (68.4)
Age (years, mean)	49	52
smokers (current+former, %)	240, 68.8	70, 71.4
FEV_1 (% pred., mean $\pm$ SD)	66.3 $\pm$ 30.2	76.6 $\pm$ 29.2
FEV_1/FVC (mean $\pm$ SD)	0.78 $\pm$ 0.22	0.83 $\pm$ 0.19*
AAT (mg/dL, mean $\pm$ SD)	130.5 $\pm$ 37.4	96.9 $\pm$ 27.6**

*$p = 0.009$.
**$p < 0.001$.

For further analysis, and to increase the sample size, we merged PI*MS and PI*MZ individuals in the ''intermediate AATD'' category. We are aware that the risk for COPD varies between PI*MS and PI*MZ subjects, since the latter genotype is at a higher risk than the former.[11,12] Nevertheless, since the pooled (PI*MZ+PI*MS) plasma AAT level was significantly lower (96.9 $\pm$ 27.6 mg/dL, $p < 0.001$) than that of PI*MM individuals (130.5 $\pm$ 37.4 mg/dL), we decided to consider the subjects with intermediate AATD as a single category. This agrees with the evidence that the risk for COPD is negatively correlated with plasma AAT level.[7]

The clinical characteristics of subjects diagnosed with lung disease are described in Table 3, whereas the stratification of the subjects according to GOLD criteria[10] is reported in Table 4. Finally, the characteristics of subjects investigated for the purpose of building a pedigree are summarised in Table 5.

Discussion

Although the programme concerns a rare condition, AATD, it was highly successful, as testified by the 534 physicians who shipped at least one sample for diagnosis. Nevertheless, compliance regarding the completeness in filling in the questionnaire that physicians were asked to ship back with the sample was disappointing. Forty-five percent of questionnaires submitted for lung disease and even 67% of those submitted for family screening could not be used for the purposes of this paper because of missing information.

We observed that physicians taking part in the programme were compliant with the guidelines to investigate subjects at risk for AATD,[4] although more than 50% of samples submitted for lung disease actually belonged to non-obstructed subjects. This aspect, however, cannot be considered as a negative one as discussed below.

The large majority of subjects (62%) carried the normal PI*MM genotype followed by those carrying the intermediate AATD genotype PI*MZ (17.9%) (Table 1).

Regarding clinical characteristics of subjects diagnosed with lung disease (Table 3), the PI*MM individuals and the intermediate group did not differ substantially in terms of gender prevalence, mean age, smoking habit, and FEV_1% predicted. The only significant difference, besides plasma AAT levels, was that subjects with intermediate AAT deficiency showed a slightly better ($p = 0.009$) FEV_1/FVC than PI*MM subjects. The mean age of both groups was 49 and 52 years, respectively (early onset COPD), and in both groups about 30% of subjects claimed that they never smoked (absence of detectable risk factors). Lack of a significant difference between the two groups brings to light an intriguing observation. In fact, subjects with lung disease carrying intermediate AATD can be considered a homogeneous group of subjects with common chronic lung disease and/or symptoms in which the supposed genetic component has been identified in the carriage of the AAT alleles Z or S.[13] Thus, the large group of individuals carrying the PI*MM genotype investigated for lung disease share with the intermediate AATD group, a relatively young age and a high proportion never smoked. In other words, this could be a group of individuals in which genetic susceptibility to cigarette smoke or other environmental factors is amplified. Individuals with such characteristics would represent an ideal cohort for investigating susceptibility genes other than the AAT gene[14,15]; their relatively young age also implies the likelihood of finding living parents, which is an usual barrier in designing genetic investigations in older COPD individuals.

More interesting was the stratification of the subjects according to GOLD criteria.[10] In both PI*MM and intermediate AATD, 50% and 59%, respectively, of the subjects belonged to the at risk stage 0. The two most severe GOLD stages (3 and 4) comprised 33% and 24% of the total

Table 4 Stratification of subjects screened for lung disease according to GOLD classification and corresponding plasma AAT levels.

	PI*MM		Intermediate deficiency (PI*MS+PI*MZ)	
	N (%)	Plasma AAT (mg/dL, mean+SD)	*N* (%)	Plasma AAT (mg/dL, mean+SD)
Stage 0	175 (50)	130.1±34.0	58 (59)	91.2±25.4*
Stage 1	9 (2.5)	115.1±41.9	5 (5)	113.7±17.4
Stage 2	49 (14)	132.5±38.4	11 (11)	91.8±24.7
Stage 3	61 (17.5)	128.0±32.4	17 (17)	106.1±31.6
Stage 4	55 (16)	135.1±42.1	7 (7)	118.7±28.8
Stage 1→4	174	130.9±38.1	40	105.2±28.9**

All the other comparisons are not significant.
*$p<0.000001$ vs MM.
**$p=0.04$ vs MM.

Table 5 Clinical phenotype of subjects investigated for family screening.

	PI*MM		Intermediate AATD (PI*MS+PI*MZ)	
	Healthy 37/50 (74%)	Lung disease 13/50 (26%)	Healthy 75/91 (82%)	Lung disease 16/91 (18%)
Male (%)	46	46	36	50
Age (years, mean)	38	38	32	37
Smokers (current+former, %)	28	65	37	43
FEV$_1$ (% pred., mean±SD)	113.2±15.4	92±26.2	113±13.2	99±19.7
FEV$_1$/FVC (mean±SD)	1.01±0.06	0.89±0.15	1.02±0.07	0.92±0.12
AAT (mg/dL, mean±SD)	138±38	145±21	94±21	101±25

subjects, respectively (Table 4). Although the largest subsets of subjects were non-obstructed subjects with chronic bronchitis symptoms (cough and phlegm), this category, also referred to as stage 0, is no longer included as a COPD stage,[16] since the risk of progression towards airflow obstruction is not clearly defined. Nevertheless, the GOLD document emphasises the fact that the public health message about this abnormal condition is unchanged. We believe that a secondary, yet important output, of a targeted detection programme for AATD is the enrolment of subjects potentially at risk for developing COPD or in its very early stages. Several aspects of the programme, including information collection, blood sampling, and reporting of the genetic analysis results, are useful to reinforce the need for smoking cessation and other preventive tools in subjects, irrespective of their genotype, with a high likelihood of succeeding in changing the natural history of their lung health if they modify their lifestyle.

An interesting feature was observed when the plasma AAT levels were considered according to this stratification. When the mean level of AAT in subjects with intermediate AATD in stage 0 and the obstructed type (stage 1→4) was compared with that of the parallel groups of PI*MM subjects, the level of significance dropped from <0.000001 to 0.04. Our data show that non-obstructed subjects with intermediate AATD have plasma levels of AAT lower than that of pooled obstructed patients. One possible explanation for this behaviour is that AAT is a well-known acute phase reactant.[17] In this setting, we may postulate that the increased AAT plasma concentration would be correlated with the systemic inflammation which is present in COPD. This observation also correlates with the elevated C reactive protein (CRP) in COPD subjects.[18,19] An intrinsic consideration for AATD diagnosis lies in the fact that the plasma level of AAT is usually the first, crucial parameter evaluated, upon this value further diagnostic procedures are then decided. It would be important to carefully design the confidence intervals for each genotypic class, in order not to misdiagnose subjects with intermediate AATD. These data need confirmation and further investigation in larger cohorts.

A final consideration is reserved for subjects investigated for family screening purposes (Table 5). As expected, in both PI*MM and intermediate AATD groups, the majority of individuals (74% and 82%, respectively) were healthy, especially if they did not smoke.[4] Nevertheless, PI*MZ individuals detected by family screening should be carefully monitored, since it has been reported that, at variance with PI*MZ individuals detected on the basis of respiratory symptoms, they are the only group at risk for hospital admission for COPD.[20]

In conclusion, programmes for targeted detection of AATD, such as the one currently available in Italy, yield secondary outputs that should not be disregarded. First of all, they confirm the importance of the important public

health warning regarding the adverse effects of smoking directed at young individuals in very early stages of obstructive disease. These subjects are likely to change the natural history of their lung health if they change their lifestyle. A second point of interest is the possibility of enrolling smokers in whom susceptibility to environmental factors for COPD is particularly marked. They would represent cohorts of ideal individuals for genetic investigation. Finally, we noticed an interesting trend in plasma level of AAT in obstructed subjects with intermediate AATD different from that in non-obstructed cases; these findings have prompted further investigations.

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Conflict of interest statement

All authors have no financial or personal relationship with other people or organisation that could inappropriately influence their work. No part of the research has been funded by tobacco industry sources.

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